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(54) Title: MONOCLONAL ANTIBODIES AND THEIR USE

(57) Abstract

Monoclonal antibodies to the genus *Streptococcus*, the labelled antibodies, compositions and kits containing them, and their use in diagnosis of antigen and treatment.

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MONOCLONAL ANTIBODIES AND THEIR USE

BACKGROUND OF THE INVENTION

Of current interest in the fields of analysis and diagnosis is the use of monoclonal antibodies to determine the presence of antigens or species in specimens such as urine, blood, water, milk, and the like.

More particularly, monoclonal antibodies specific for the antigens or species of *Streptococci* are desired which when used will rapidly diagnose the presence of such organisms in specimens.

Divisions have been made among the Streptococcus species. Some of the representative members include Streptococcus pneumoniae, S. pyogenes, S. agalactiae, S. bovis, S. cremoris, S. dysgalactiae, S. fecalis, S. faecium, S. lactis, S. equisimilis, S. zooepidermicus, S. equi, and S. uberis.

Additionally, the Streptococci have been differentiated on the basis of their hemolytic characteristics. The Lancefield nomenclature classifies the beta Streptococci primarily upon immunological methods for their differentiation. In particular, Lancefield has made use of the precipitin test to show the presence of both group-specific and type-specific antigens in

the hemolytic Streptococci. Presently, there are approximately seven groups (A to G) of beta Streptococci which correlate the biochemical homogeneity of the immunological groups with the natural habitat of the bacteria.

While involved with very different disease processes from the beta hemolytic Streptococci, S. pneumoniae is nevertheless included within the broad grouping of Streptococcal organisms. Many different classification systems have been utilized for the Streptococcus pneumoniae. The American and the Danish classifications are two of the better known systems, with the American system based on the order of discovery, and the Danish system in order of families. However, both recognize the same number of types; 83.

There are a variety of toxic substances that are produced by Streptococci, including hemolysins, fibrinolysins, leucocidin, and erythrogenic toxins. The erythrogenic toxin is a substance, which upon intradermal injection in man, gives rise to a marked local erythema. This toxin is antigenic, and relatively heat-resistant. Further, at least two types of filterable hemolysins are formed by Streptococci; Streptolysin S, which is sensitive to heat and

acid, and Streptolysin O, which is sensitive to oxygen, but resistant to heat and acid.

Few, if any, pathogenic microorganisms can lay claim to wider or more multifarious activities than the Streptococci. The Streptococci will be described with particular reference to beta Streptococcus Group A, as it is one of the most deadly and lethal microorganisms to man. It is the common cause of skin infections, producing a rapidly spreading cellulitus that may enter the blood stream and produce blood poisoning. Other diseases commonly caused by the beta Streptococcus Group A include strep throat, tonsillitis, streptococcal pneumonia, and all wound infections where severe results may ensue without prompt treatment. The diagnosis of Streptococcal infection in tissues may be sometimes difficult because the organism may not be identified. The use of a rapid specific diagnostic agent to detect the Streptococci would be of extreme utility under these circumstances. Additionally, rheumatic fever is a regular accompaniment of Streptococcal infections. It appears to be an immune reaction to the organism. It occurs days or weeks after the infection. The most common cause of valvular heart disease

remains the beta Streptococcus Group A. There are several dozen types of beta Streptococcus Group A distinguished by different surface properties; i.e., carbohydrates of Lancefield's groups, proteins, T antigens, and R antigens. A small number of these may produce a severe kidney disease called glomerulonephritis. A somewhat larger number have been associated with rheumatic fever. The utilization of the product in a diagnostic kit for throat infection would be used frequently, as sore throat or upper respiratory infections occur an average of six or seven times per year in children of school age. An immediate diagnostic that would give "same day" diagnosis, rather than to wait two to three days before treatment, would improve the management of these cases and provide more specific therapy. The ability of monoclonal antibodies specifically to bind to antigens of Streptococci can provide many opportunities for diagnosis and treatment. Such specificity is a most important requirement for proper and accurate analysis and/or diagnosis, particularly in diagnosing the presence of diseases such as Streptococci which require prompt treatment.

A wide variety of isotopic and nonisotopic

immunoassays have been utilized in conjunction with monoclonal antibodies to test for the presence of an antigenic substance. At the present time, agglutination, immuno-fluorescent, chemiluminescent or fluorescent immunoassay, immuno-electron microscopy, radiometric assay systems, radio immunoassays, and enzyme-linked immunoassays are the most common techniques used with the monoclonal antibodies. Other techniques include bioluminescent, fluorescence polarization, and photon-counting immunoassays.

When utilizing the enzyme-linked immunoassay procedure (EIA), it is necessary to bind, or conjugate, the monoclonal antibody with an enzyme capable of functioning in such assay; such as alkaline phosphatase.

The enzyme-linked monoclonal antibody can then be used in the known enzyme-linked immunosorbent assay procedure to determine the presence of an antigenic substance.

After the specific antigen is identified, the serotype of the infecting organism can be determined, and appropriate treatment can then be initiated to rapidly and efficiently eliminate the disease.

The production of monoclonal antibodies

is now a well-known procedure first described by Kohler and Milstein (Eur. J. Immunol. 6, 292 (1975)). While the general technique of preparing hybridomas and the resultant monoclonal antibodies is understood, it has been found that preparing a specific monoclonal antibody to a specific antigen is difficult, mainly due to the degree of specificity and variations required in producing a particular hybridoma.

SUMMARY OF THE INVENTION

The present invention provides novel monoclonal antibodies for use in accurately and rapidly diagnosing samples for the presence of Streptococci antigens and/or organisms.

Briefly stated, the present invention comprises monoclonal antibodies specific for an antigen or species of Streptococci; in particular, the antigens or species of Streptococcus pneumoniae, S. pyogenes, S. agalactiae, S. bovis, S. cremoris, S. dysgalactiae, S. fecalis, S. faecium, S. lactis, S. equisimilis, S. zooepidermicus, S. equi, S, uberis, the antigens or species of beta Streptococcus (specifically Groups A through G), the antigen or antigens to the toxins of Streptococci, such as the erythrogenic toxin and the streptolysins O and S, as well as a

monoclonal antibody broadly cross-reactive with an antigen for each species of the genus Streptococcus.

The invention also comprises labeled monoclonal antibodies for use in diagnosing the presence of the Streptococci antigens, each comprising a monoclonal antibody against one of the above-mentioned antigens to Streptococci or to a particular species thereof and linked thereto an appropriate label. The label can be chosen from the group consisting of a radioactive isotope, enzyme, fluorescent compound, chemiluminescent compound, bioluminescent compound, ferromagnetic atom, or particle, or any other label.

The invention further comprises the process for diagnosing the presence of Streptococci antigens or organisms in a specimen comprising contacting said specimen with the labeled monoclonal antibody in an appropriate immunoassay procedure.

Additionally, the invention is also directed to a therapeutic composition comprising a monoclonal antibody for an antigen of Streptococcus and a carrier or diluent, as well as kits containing at least one labeled monoclonal antibody

to an antigen or a Streptococcus.

DETAILED DESCRIPTION

The monoclonal antibodies of the present invention are prepared by fusing spleen cells, from a mammal which has been immunized against the particular Streptococcus antigen, with an appropriate myeloma cell line, preferably NS0 (uncloned), P3NS1-Ag4/1, or Sp2/0 Ag14. The resultant product is then cultured in a standard HAT (hypoxanthine, aminopterin, and thymidine) medium. Screening tests for the specific monoclonal antibodies are employed utilizing immunoassay techniques which will be described below.

The immunized spleen cells may be derived from any mammal, such as primates, humans, rodents (i.e., mice, rats, and rabbits), bovine, ovine, canine, or the like, but the present invention will be described in connection with mice. The mouse is first immunized by injection of the particular Streptococcus antigen chosen generally for a period of approximately eleven weeks. When the mouse shows sufficient antibody production against the antigen, as determined by conventional assay, it is given a booster injection of the appropriate Streptococcus antigen, and then killed so that the immunized spleen may

be removed. The fusion can then be carried out utilizing immunized spleen cells and an appropriate myeloma cell line.

The fused cells yielding an antibody which give a positive response to the presence of the particular Streptococcus antigen are removed and cloned utilizing any of the standard methods. The monoclonal antibodies from the clones are then tested against standard antigens to determine their specificity for the particular Streptococcus antigen. The monoclonal antibody selected, which is specific for the particular Streptococcus antigen or species, is then bound to an appropriate label.

Amounts of antibody sufficient for labeling and subsequent commercial production are produced by the known techniques, such as by batch or continuous tissue culture or culture in vivo in mammals, such as mice.

The monoclonal antibodies may be labeled with a multitude of different labels, such as enzymes, fluorescent compounds, luminescent compounds, radioactive compounds, ferromagnetic labels, and the like. The present invention will be described with reference to the use of an enzyme labeled monoclonal antibody. Some

of the enzymes utilized as labels are alkaline phosphatase, glucose oxidase, galactosidase, peroxidase, or urease, and the like.

Such linkage with enzymes can be accomplished by any one of the conventional and known methods, such as the Staphylococcal Protein A method, the glutaraldehyde method, the benzoquinone method, or the periodate method.

Once the labeled monoclonal antibody is formed, testing is carried out employing one of a wide variety of conventional immunoassay methods. The particular method chosen will vary according to the monoclonal antibody and the label chosen. At the present time, enzyme immunoassays are preferred due to their low cost, reagent stability, safety, sensitivity, and ease of procedure. One example is enzyme-linked immunosorbent assay (EIA). EIA is a solid phase assay system which is similar in design to the radiometric assay, but which utilizes an enzyme in place of a radioactive isotope as the immunoglobulin marker.

Fluorescent-immunoassay is based on the labeling of antigen or antibody with fluorescent probes. A nonlabeled antigen and a specific antibody are combined with identical fluorescently

labeled antigen. Both labeled and unlabeled antigen compete for antibody binding sites. The amount of labeled antigen bound to the antibody is dependent upon, and therefore a measurement of, the concentration of nonlabeled antigen. Examples of this particular type of fluorescent-immunoassay would include heterogenous systems such as Enzyme-Linked Fluorescent Immunoassay, or homogeneous systems such as the Substrate Labeled Fluorescent Immunoassay. The most suitable fluorescent probe, and the one most widely used is fluorescein. While fluorescein can be subject to considerable interference from scattering, sensitivity can be increased by the use of a fluorometer optimized for the probe utilized in the particular assay and in which the effect of scattering can be minimized.

In fluorescence polarization, a labeled sample is excited with polarized light and the degree of polarization of the emitted light is measured. As the antigen binds to the antibody its rotation slows down and the degree of polarization increases. Fluorescence polarization is simple, quick, and precise. However, at the present time its sensitivity is limited to the micromole per liter range and upper nano-

mole per liter range with respect to antigens in biological samples.

Luminescence is the emission of light by an atom or molecule as an electron is transferred to the ground state from a higher energy state. In both chemiluminescent and bioluminescent reactions, the free energy of a chemical reaction provides the energy required to produce an intermediate reaction or product in an electronically excited state. Subsequent decay back to the ground state is accompanied by emission of light. Bioluminescence is the name given to a special form of chemiluminescence found in biological systems, in which a catalytic protein or enzyme, such as luciferase, increases the efficiency of the luminescent reaction. The best known chemiluminescent substance is luminol.

A further aspect of the present invention is a therapeutic composition comprising one or more of the monoclonal antibodies to the particular Streptococcus antigen or species, as well as a pharmacologically acceptable carrier or diluent. Such compositions can be used to treat humans and/or animals afflicted with some form of Streptococci infections and they are used in amounts effective to cure; an amount

which will vary widely dependent upon the individual being treated and the severity of the infection.

One or more of the monoclonal antibodies can be assembled into a diagnostic kit for use in diagnosing for the presence of an antigen, antigens, or species of Streptococcus in various specimens. It is also possible to use the broadly cross-reactive monoclonal antibody which can identify the genus Streptococcus alone or as part of a kit containing antibodies that can identify other bacterial genera or species of Streptococci and/or other bacteria.

In the past there have been difficulties in developing rapid kits because of undesirable cross-reactions of specimens with antiserum. The use of monoclonal antibodies can eliminate these problems and provide highly specific and rapid tests for diagnosis. A rapid and precise kit could replace or augment existing tests and permit early direct therapy using precise antibiotics. Avoiding multiple antibiotics or more expensive or hazardous antibiotics would represent substantial patient and hospital savings. Additionally, a kit can be used on an out-patient basis. At present the lack of a

rapid test giving "same day" answers may delay the initiation of treatment until the patient has developed more severe symptoms or may require the initiation of more costly therapy in a sick patient. A test that would return results within an hour or two would be a substantial convenience to patients.

In addition to being sold individually, the kit could be included as a component in a comprehensive line of compatible immunoassay reagents sold to reference laboratories to detect the species and serotypes of Streptococci.

One preferred embodiment of the present invention is a diagnostic kit comprising at least one labeled monoclonal antibody against a particular Streptococcus antigen or species, as well as any appropriate stains, counterstains, or reagents. Further embodiments include kits containing at least one control sample of a Streptococcus antigen and/or a cross-reactive labeled monoclonal antibody which would detect the presence of any of the Streptococci organisms in a particular sample. Specific antigens to be detected in this kit include the common antigens of Streptococci, the antigens of S. pneumoniae, S. pyogenes, S. agalactiae, S. bovis,

S. cremoris, S. dysgalactiae, S. fecalis, S. faecium, S. lactis, S. equisimilis, S. zooepidermicus, S. equi, S. uberis, the antigens of beta Streptococcus, as well as the antigens to the toxins of Streptococci.

Monoclonal diagnostics which detect the presence of Streptococci antigens can also be used in periodic testing of water sources, food supplies and food processing operations. Thus, while the present invention describes the use of the labeled monoclonal antibodies to determine the presence of a standard antigen, the invention can have many applications in diagnosing the presence of antigens by determining whether specimens such as urine, blood, stool, water, milk, and the like contain the particular Streptococcus antigen. More particularly, the invention could be utilized as a public health and safety diagnostic aid, whereby specimens such as water or food could be tested for possible contamination.

The invention will be further illustrated in connection with the following examples which are set forth for purposes of illustration only and not by way of limitation.

The monoclonal antibodies of the present invention were prepared generally according to the method of Kohler and Milstein, supra.

In the Examples:

API = Analytical Profile Index (ref. Ayerst Laboratories)

DMEM = Dulbeccos Modified Eagles Medium

FCS = Foetal Calf Serum

% T refers to vaccine concentrations measured in a 1 cm light path

PBS = Phosphate Buffered Saline

TSB = Tryptone Soya Broth

CFA = Complete Freunds Adjuvant

Example 1

A. Antigen Preparation

Streptococcus antigen was obtained from the National Collection of Type Cultures and tested by standard biochemical methods of microbial identification to confirm its identity (using API profiles). The Streptococcus was removed from the lyophile, grown on blood agar, and tested by API to confirm its identity and purity. The bacteria were transferred for growth on to TSB and harvested for use as a source of antigen. The organisms were boiled and washed in formed saline by repeated centrifugation, and they were then resuspended in 1% formol saline.

B. Animal Immunisation

Balb/c mice were injected with the prepared antigen. They were given one intraperitoneal injection per week for three weeks (0.05 ml 80% T vaccine), followed by two intravenous injections after intervals of 1 and 2 weeks. The mice were bled approximately six days after the last injection and the serum tested for antibodies by assay. The conventional assay used for this serum titer testing was the enzyme-linked immunosorbent assay system. When

the mice showed antibody production after this regimen, generally a positive titer of at least 10,000, a mouse was selected as a fusion donor and given a booster injection (0.05 ml 80% T vaccine) intraperitoneally, three days prior to splenectomy.

C. Cell Fusion

Spleen cells from the immune mice were harvested three days after boosting, by conventional techniques. First, the donor mouse selected was killed and surface-sterilised by immersion in 70% ethyl alcohol. The spleen was then removed and immersed in approximately 2.5 ml DMEM to which had been added 3% FCS. The spleen was then gently homogenised in a LUX homogenising tube until all cells had been released from the membrane, and the cells were washed in 5 ml 3% FCS-DMEM. The cellular debris was then allowed to settle and the spleen cell suspension placed in a 10 ml centrifuge tube. The debris was then rewashed in 5 ml 3% FCS-DMEM. 50 ml suspension were then made in 3% FCS-DMEM.

The myeloma cell line used was NS0 (uncloned), obtained from the MRC Laboratory of Molecular Biology in Cambridge, England. The myeloma cells were in the log growth phase, and rapidly dividing. Each cell line was washed using, as tissue culture medium, DMEM containing 3% FCS.

The spleen cells were then spun down at the same time that a relevant volume of myeloma cells were spun down (room temperature for 7 minutes at 600 g), and each resultant pellet was then separately resuspended in 10 ml 3% FCS-DMEM. In order to count the myeloma cells, 0.1 ml of the suspension was diluted to 1 ml and a haemacytometer with phase microscope was used. In order to count the spleen cells, 0.1 ml of the suspension was diluted to 1 ml with Methyl Violet-citric acid solution,

and a haemacytometer and light microscope were used to count the stained nuclei of the cells.

10^8 spleen cells were then mixed with 5 x 10^7 myeloma cells, the mixture washed in serum-free DMEM high in glucose, and centrifuged, and all the liquid removed. The resultant cell pellet was placed in a 37°C water-bath. 1 ml of a 50 w/v solution of polyethylene glycol 1500 (PEG) in saline Hepes, pH approximately 7.5, was added, and the mixture gently stirred for approximately 1.5 minutes. 10 ml serum-free tissue culture medium DMEM were then slowly added, followed by up to 50 ml of such culture medium, centrifugation and removal of all the supernatant, and resuspension of the cell pellet in 10 ml of DMEM containing 18% by weight FCS.

10 µl of the mixture were placed in each of 480 wells of standard multiwell tissue culture plates. Each well contains 1.0 ml of the standard HAT medium (hypoxanthine, aminopterin and thymidine) and a feeder layer of Balb/c macrophages at a concentration of 5 x 10^4 macrophages/well.

The wells were kept undisturbed and cultured at 37°C in 9% CO_2 air at approximately 100% humidity. The wells were analysed for growth, utilising the conventional inverted microscope procedure, after about 5 to 10 days. In those wells in which growth was present in the inhibiting HAT medium, screening tests for the specific monoclonal antibody were made utilising the conventional enzyme immunoassay screening method described below.

D. <u>Cloning</u>

From those wells which yielded antibody against the antigen, cells were removed and cloned using the limiting dilution method. In limiting dilution, dilutions of cell suspensions in 18% FCS-DMEM + Balb/c mouse macrophages were made to achieve one cell/well and one-half cell/well

in a 96-well microtitre plate. The plates were incubated for 7-14 days at 37 C, 97% RH, 7-9% CO_2 until semi-confluent. The supernatants were assayed for specific antibody by the standard enzyme immunoabsorbent assay.

The clones were assayed by the enzyme immunoassay method to determine antibody production.

E. Monoclonal Selection

The monoclonal antibodies from the clones were screened by the standard techniques for binding to the antigen, prepared as in the immunisation, and for specificity in a test battery of Streptococcus species and related genera bearing different antigens. Specifically, a grid of microtitre plates containing a representative selective of Streptococcus organisms was prepared, boiled, and utilised as a template to define the specificity of the parent group. The EIA immunoassay noted above may be used.

F. Antibody Production and Purification

Balb/c mice were primed with pristane, for at least 7 days, and injected intraperitoneally with 10^7 cells of the monoclonal antibody-producing line. The ascitic fluid was harvested when the mice were swollen with fluid but still alive. The cells were then centrifuged at 1200 g for approximately 10 minutes, the cells discarded, and the antibody-rich ascites collected and stored at -20 C.

Purification was accomplished using the ammonium sulphate/DEAE method. Specifically, 10 ml of the ascites fluid were filtered through glass wool and centrifuged at 30,000 g for 10 minutes. The ascites was then stirred at +4°C, and an equal volume of cold, saturated ammonium sulphate added slowly. The mixture was stirred for 30 minutes after the addition was complete. The precipitate was harvested by centrifugation at 10,000 g for 10 minutes. The precipitate was dissolved in a minimum

volume of cold phosphate/EDTA buffer (20 mM sodium phosphate, 10 mM EDTA, pH 7.5, + 0.02% sodium azide). The solution was dialysed versus 2x1000 ml of the same buffer, at +4°C. The dialysed, redissolved precipitate was centrifuged at 30,000 g for 10 minutes and applied to a 10 ml column of DEAE-cellulose, previously equilibrated in phosphate/EDTA buffer. The monoclonal antibody was eluted with phosphate/EDTA buffer.

G. Enzyme-Monoclonal Linkage

The monoclonal antibody specific against the antigen, prepared as above, was linked to an enzyme, viz. highly-purified alkaline phosphatase, using the one-step glutaraldehyde method. Monoclonal antibody was dialysed with alkaline phosphatase (Sigma Type VII-T) against 2 x 1000 ml of PBS pH 7.4, at +4°C. After dialysis, the volume was made up to 2.5 ml with PBS and 25 µl of a 20% solution of glutaraldehyde in PBS was added. The conjugation mixture was left at room temperature for 1.5 hours. After this time, glutaraldehyde was removed by gel filtration on a Pharmacia PD-10 (Sephadex G-25M) column, previously equilibrated in PBS. The conjugate was eluted with 3.5 ml PBS and then dialysed against 2 x 2000 ml of Tris buffer (50 mM Tris, 1 mM magnesium chloride, pH 8.0 plus 0.02% sodium azide) at +4°C. To the dialysed conjugate was added 1/10th its own volume of 10% BSA in Tris buffer. The conjugate was then sterile-filtered through a 0.22 µm membrane filter into a sterile amber vial, and stored at +4°C.

H. Monoclonal Antibody Conjugate Testing

The enzyme immunoassay method was used for testing. This method comprises coating the wells of a standard polyvinyl chloride (PVC) microtitre tray with the antigen, followed by addition of monoclonal antibody enzyme conjugate, and finally addition of the enzyme substrate, para-nitrophenyl phosphate.

If deemed necessary, the particular epitopic site to which the antibody attaches to the antigen can also be determined. The same enzyme immunoassay method can also be used to determine whether diagnostic specimens such as urine, blood, stool, water or milk contain the antigen. In such cases, the antibody can first be bound to the plate.

Example 2

The procedure of Example 1 was repeated, to give a monoclonal antibody broadly cross-reactive with each species of the genus Streptococcus.

Tests using the present invention are superior to existing tests, based on the following advantages: (i) greater accuracy; (ii) same day results, within an hour or two; (iii) reduction in amount of skilled labour required to administer laboratory procedures, resulting in reduced labour costs; (iv) reduction in laboratory time and space used in connection with tests, resulting in reduced overhead expenses; and (v) improved therapy based upon early, precise diagnosis.

While the invention has been described in connection with certain preferred embodiments, it is not intended to limit the scope of the invention to the particular form set forth but, on the contrary, it is intended to cover such alternatives, modifications, and equivalents as may be included within the spirit and scope of the invention as defined by the appended claims.

CLAIMS

1. A monoclonal antibody specific to the antigen or antigens of Streptococcus agalactiae.

2. A monoclonal antibody specific to the antigen or antigens of Streptococcus bovis.

3. A monoclonal antibody specific to the antigen or antigens of Streptococcus cremoris.

4. A monoclonal antibody specific to the antigen or antigens of Streptococcus dysgalactiae.

5. A monoclonal antibody specific to the antigen or antigens of Streptococcus fecalis.

6. A monoclonal antibody specific to the antigen or antigens of Streptococcus faecium.

7. A monoclonal antibody specific to the antigen or antigens of Streptococcus lactis.

8. A monoclonal antibody specific to the antigen or antigens of Streptococcus equisimilis.

9. A monoclonal antibody specific to the antigen or antigens of Streptococcus zooepidemicus.

10. A monoclonal antibody specific to the antigen or antigens of Streptococcus equi.

11. A monoclonal antibody specific to the antigen or antigens of Streptococcus uberis.

12. A monoclonal antibody specific to the antigen or antigens of beta Streptococci Groups E, F and/or G.

13. A monoclonal antibody specific to the common antigens of Streptococci.

14. A monoclonal antibody specific to the antigen or antigens of the toxins of Streptococci.

15. A monoclonal antibody specific to the antigen or antigens of the erythrogenic toxins of Streptococci.

16. A monoclonal antibody specific to the antigen or antigens of the streptolysin S toxins of Streptococci.

17. A monoclonal antibody specific to the antigen or antigens of the streptolysin O toxins of Streptococci.

18. A monoclonal antibody broadly cross-reactive with an antigen of all species of the genus Streptococcus.

19. A monoclonal antibody according to any of claims 1 to 18, which is labelled.

20. A monoclonal antibody according to claim 19, wherein the label is a radioactive isotope, enzyme, fluorescent compound, bio-luminescent compound, chemi-luminescent compound, or ferromagnetic atom or particle.

21. A monoclonal antibody according to claim 20, wherein the label is an enzyme capable of being used in an enzyme-linked immunoassay procedure, a fluorescent compound or probe capable of being used in an immuno-fluorescent, fluorescent, enzyme-fluorescent, fluorescence-polarisation or photon-counting immunoassay procedure, a chemi-luminescent compound capable of being used in a luminescent or enzyme-linked immunoassay procedure, or a bio-luminescent compound capable of being used in a bio-luminescent immunoassay procedure.

22. A monoclonal antibody according to claim 20, wherein the label is an enzyme selected from alkaline phosphatase, glucose oxidase, galactosidase and peroxidase, fluorescein, a chemi-luminescent compound selected from luminol and luminol derivatives, or a bio-luminescent compound selected from luciferase and luciferase derivatives.

23. A monoclonal antibody according to any preceding claim, for use in treating Streptococcus infections.

24. A process for diagnosing for the presence of an antigen of Streptococcus in a specimen, which comprises contacting the specimen with a monoclonal antibody according to any of claims 20 to 23 in an immunoassay procedure appropriate to the label.

25. A therapeutic composition which comprises a monoclonal antibody according to any of claims 1 to 23 and a pharmaceutically-acceptable carrier or diluent.

26. A kit for diagnosing for the presence of a gram-negative bacterial infection, which comprises a monoclonal antibody according to any of claims 1 to 23 and, as a control, a known Streptococcus antigen.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 86/00412

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: C 07 K 15/00; C 12 P 21/00; G 01 N 33/577; 33/569; A 61 K 39/40 // C 12 N 15/00 (C 12 P 21/00; C 12 R 1:91)

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	G 01 N; C 12 P; A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Chemical Abstracts, vol. 104, no. 1,, 6 January 1986 (Columbus, Ohio, US) R.A. Polin et al.: "Monoclonal antibodies against group B streptococcus", see page 399, abstract no. 4311r, & Monoclonal Antibodies Bact., 1985, 1,37-58 (Eng.)	1
Y		2-26
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Y	Chemical Abstracts, vol. 102, no. 11, 18 March 1985 (Columbus, Ohio, US) N.S. Greenspan et al.: "Serologic and topographic characterization of idiotopes on murine monoclonal antistreptococal group A carbohydrate antibodies", see page 445, abstract no. 94091e, & J. Immunol., 1985, 134(2), 1065-1072 (Eng.)	1-26
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Y	Chemical Abstracts, vol. 100, no. 19, 7 May 1984 (Columbus, Ohio, US) H. Herbst et al.: "Grouping of hemolytic	./.

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
10th October 1986	21 NOV 1986
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EUROPEAN PATENT OFFICE	M. VAN MOL

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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	streptococci by monoclonal antibodies: determinant specificity, cross-reactivity and affinity", see page 402, abstract no. 154967r, & Ann. Immunol. (Paris) 1983, 134D(3)349-371 (Eng.)	1-26
X	Clinical Chemistry, vol. 27, no. 11, November 1981 (Easton, Pennsylv., US) E. Dale Sevier: "Monoclonal antibodies in clinical immunology", pages 1797-1806, see page 1799	1
Y		2-26
Y	EP, A, 0077734 (MOLECULAR GENETICS INC.) 27 April 1983 see page 16; claims 1,27,39,42,51	1-26
Y	EP, A, 0109012 (ABBOTT LABORATORIES) 23 May 1984 see the entire document	24,26
Y	US, A, 4461829 (A.C. GREENQUIST) 24 July 1984 see claims 1-3; column 2, lines 8-40; column 10, lines 17-26 and 51	19-22,24

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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/GB 86/00412 (SA 13889)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 24/10/86

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0077734	27/04/83	AU-A- 8945482	28/04/83
		JP-A- 58099423	13/06/83
		US-A- 4443549	17/04/84
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